12-1985

Cisplatin Ototoxicity in the Guinea Pig: An Electrophysiological, Morphological, and Biochemical Study

Sarah E. Barron
East Tennessee State University

Barron, Sarah Elizabeth

CISPLATIN OTOTOXICITY IN THE GUINEA PIG: AN ELECTROPHYSIOLOGICAL, MORPHOLOGICAL, AND BIOCHEMICAL STUDY

East Tennessee State University PH.D. 1985

PLEASE NOTE:

In all cases this material has been filmed in the best possible way from the available copy. Problems encountered with this document have been identified here with a check mark ✓.

1. Glossy photographs or pages ✓
2. Colored illustrations, paper or print _____
3. Photographs with dark background ✓
4. Illustrations are poor copy _____
5. Pages with black marks, not original copy _____
6. Print shows through as there is text on both sides of page _____
7. Indistinct, broken or small print on several pages ✓
8. Print exceeds margin requirements _____
9. Tightly bound copy with print lost in spine _____
10. Computer printout pages with indistinct print _____
11. Page(s) _____ lacking when material received, and not available from school or author.
12. Page(s) _____ seem to be missing in numbering only as text follows.
13. Two pages numbered _____. Text follows.
14. Curling and wrinkled pages _____
15. Dissertation contains pages with print at a slant, filmed as received _____
16. Other _____

_____

_____

University
Microfilms
International

CISPLATIN OTOTOXICITY IN THE GUINEA PIG: AN ELECTROPHYSIOLOGICAL, MORPHOLOGICAL, AND BIOCHEMICAL STUDY

A Dissertation

Presented to

the Faculty of the Department of Pharmacology

Quillen-Dishner College of Medicine

East Tennessee State University

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

---

by

Sarah Elizabeth Barron

December, 1985

APPROVAL

This is to certify that the Graduate Committee of

SARAH ELIZABETH BARRON

met on the

16th day of November, 1983

The committee read and examined her dissertation, supervised her defense of it in an oral examination, and decided to recommend that her study be submitted to the Graduate Council and the Associate Vice-President for Research and Graduate Studies in partial fulfillment of the requirements for the degree Doctor of Philosophy in Biomedical Sciences.

Ernest A. Digneault
Chairman, Graduate Committee

Fred E. Hosler

Hugh E. Criswell

John Hamack

Donald B. Hoover

Richard A. Crofts
Associate Vice-President for
Research and Graduate Studies

Signed on behalf of
the Graduate Council

ABSTRACT

CISPLATIN OTOTOXICITY IN THE GUINEA PIG:

A ELECTROPHYSIOLOGICAL, MORPHOLOGICAL AND BIOCHEMICAL STUDY

by

Sarah E. Barron

The purpose of this study was to determine changes induced by cisplatin on hair cell (HC) morphology, compound action potential (CAP), Preyer reflex (PR), and Na,K-ATPase activity in the cochlear lateral wall.

Guinea pigs were treated with either 0.42, 0.83, 1.17, 1.50 mg/kg cisplatin, i.p. or sterile water for 12 consecutive days and tested on day 13. HC condition was visualized with scanning electron microscopy (SEM), CAP was initiated with a 1 volt click, and Na,K-ATPase activity was measured by the method of Fiske and SubbaRow.

HC damage was linear with dose and occurred throughout the turns of the cochlea with greatest predominance in the basal turn. Degrees of HC distortion were represented by separation of stereocilia, formation of blebs, and expulsion of HC contents. Complete suppression of CAP and PR occurred in all 1.5 mg/kg treated animals and 50% of the 1.17 mg/kg dose group. A 60% depression of CAP occurred in 50% of the 0.83 dose group. Na,K-ATPase activity was not significantly different between treatment groups.

These results indicated that cisplatin ototoxicity appeared to be dose-dependent for measurements of CAP, PR, and HC damage but was not related to Na,K-ATPase activity in cochlear lateral wall.

ACKNOWLEDGEMENTS

I wish to express my gratitude to Dr. Ernest A. Daigneault, my major advisor, for his invaluable guidance, teachings, and support throughout this investigation and in the preparation of this dissertation. His dedication to basic science has been an inspiration to me.

I wish also to thank the members of my committee; Dr. Donald Hoover, Dr. John Hancock, Dr. Fred Hossler and Dr. Hugh Criswell for their professionalism, constructive criticism, and expertise. Dr. Fred Hossler should be given particular recognition for his provision of working space and equipment in his personal laboratory.

Several persons, in addition to members of the committee, have contributed generously to the completion of this project. My appreciation goes to Dr. Ellen Rasch, Dr. Thomas Kwasigroch, and Dr. Frank Inman for the use of their equipment and facilities and to Sharon Hyder for her secretarial assistance.

Thanks go to my family and friends who have continually encouraged me during this endeaver. Finally, special thanks go to my husband, Rusty, who has patiently and unselfishly supported me throughout all phases of this goal. His suggestions, constructive criticisms, and assistance have been indispensable.

CONTENTS

LIST OF FIGURES

CHAPTER 1

Introduction

Background

Hearing disturbances due to the commonly used drugs quinine, salicylates, and oil of chenopodium were recognized as early as the 19th century (Hawkins, '76). However, it was not until 1945, with the introduction of streptomycin as an antitubercular agent, that functional and structural changes occurring in the cochlea were examined in detail (Hawkins, '76). Profound interest in ototoxicity became generated as streptomycin and other aminoglycosides became successfully and widely used therapeutically as antibiotics. Since this time, additional drugs have been identified as ototoxic including ethacrynic acid, furosemide, non-steroidal antiinflammatory agents, nitrogen mustard, and cisplatin.

Cisplatin, an antineosplastic agent, has recently drawn considerable interest as an ototoxic agent. One might suggest that its ototoxic effects would resemble that of other anticancer agents. Cisplatin's toxic effects on the cochlea, however, differ significantly from those of nitrogen mustard (Kisiel and Bobbin, '81). Its antitumor mechanism appears to be exclusive from its mechanism of ototoxicity (Daley-Yates and McBrien, '84). Due to

cisplatin's platinum moiety, one might also suggest that its ototoxic effects may be similar to those seen with heavy metal intoxication, but the morphological picture obtained after acute methyl mercury intoxication is quite different from that seen with cisplatin (Falk et al., 1973). Nakai et al. (1982) reported that the degeneration pattern produced by cisplatin resembled that of the aminoglycosides. While some morphological and functional changes have been identified, the mechanism of action for cisplatin's ototoxicity remains unknown.

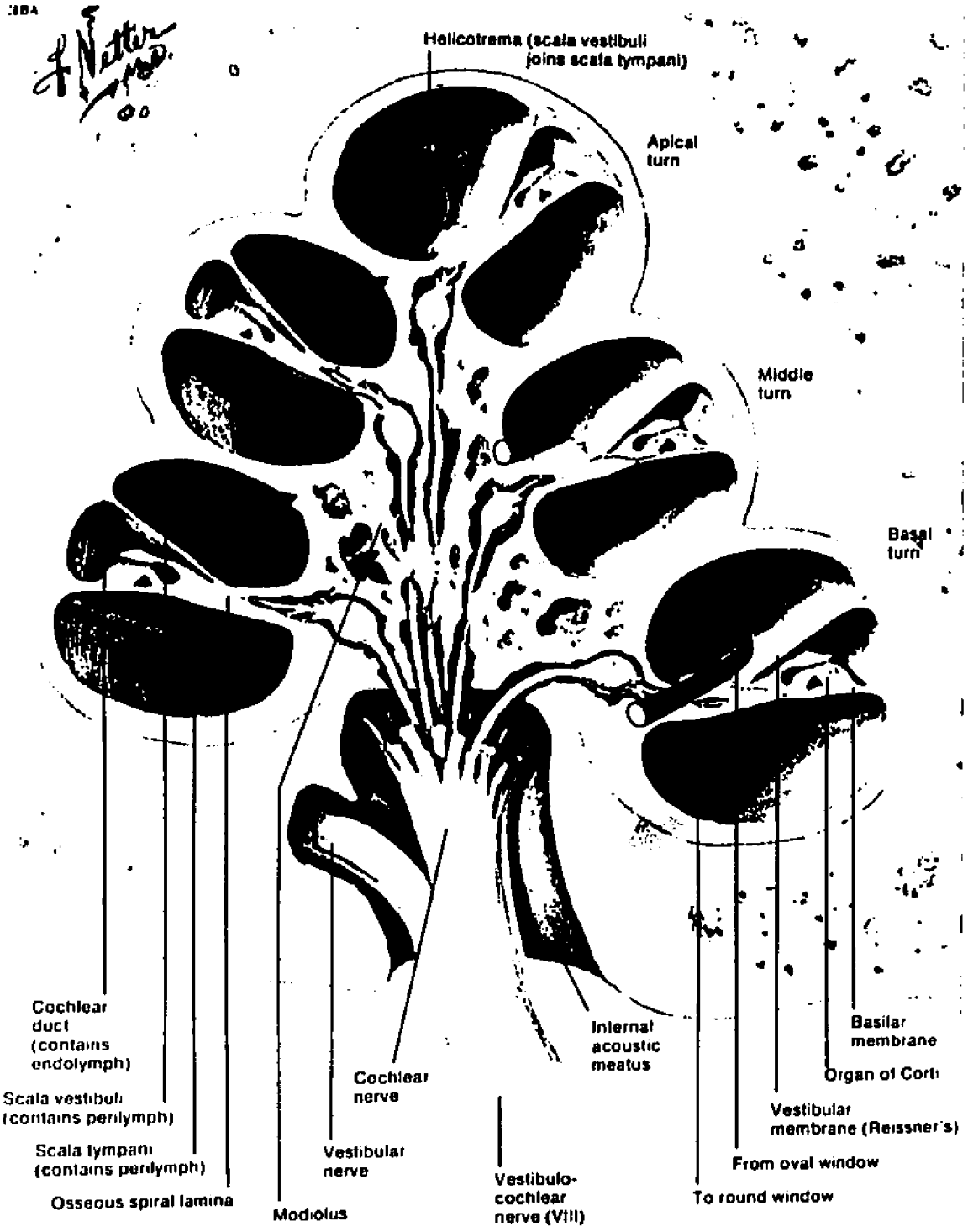
An increasing number of cancer patients are being successfully treated with cisplatin. Though not life threatening, cisplatin's toxic effects on the cochlea are of major concern. The need for a better understanding of the mechanism or mechanisms involved with the pathogenesis of cisplatin ototoxicity is currently a significant problem.

Anatomical Considerations of the Cochlea

The cochlea is composed of a membranous and osseous labyrinth which is embedded deeply in the petrous bone of the skull. The membranous labyrinth is compartmentalized into the scala vestibuli, scala media (cochlea duct), and scala tympani (fig. 1). In the scala vestibuli and scala tympani the fluid or perilymph is similar to extracellular fluid being high in sodium and low in potassium.

Figure 1

Diagram illustrating structural details within a cross section of the cochlea. Note the compartmentalization of the membranous labyrinth into the scala media (cochlea duct), scala vestibuli, and scala tympani (Netter, '70).

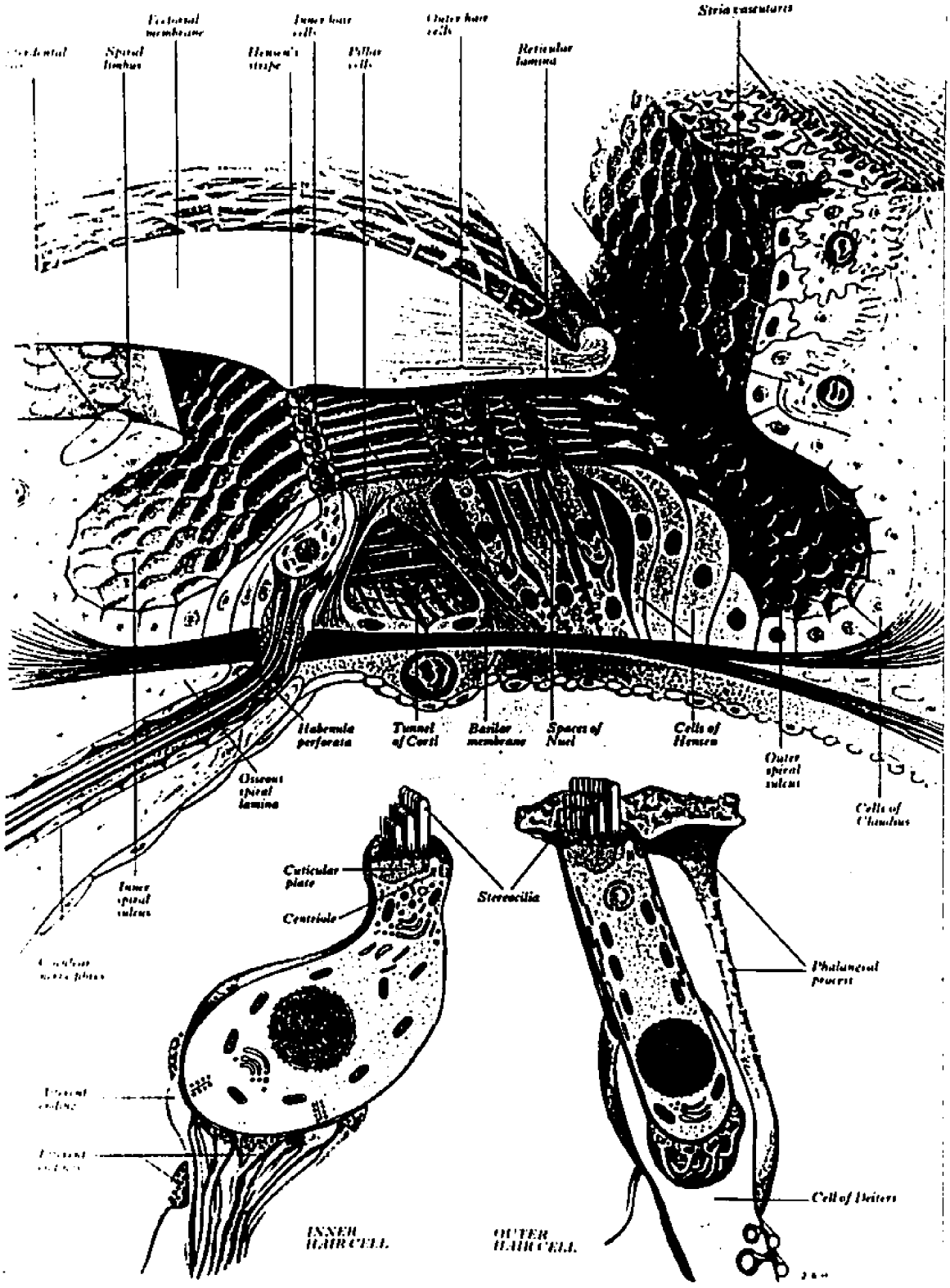

Helicotrema (scala vestibuli joins scala tympani)
Apical turn
Middle turn
Basal turn
Cochlear duct (contains endolymph)
Scala vestibuli (contains perilymph)
Scala tympani (contains perilymph)
Osseous spiral lamina
Modiolus
Vestibular nerve
Cochlear nerve
Vestibulo-cochlear nerve (VIII)
Internal acoustic meatus
To round window
From oval window
Vestibular membrane (Reissner's)
Organ of Corti
Basilar membrane

Conversely, the fluid or endolymph found in the scala media is similar to intracellular fluid having a high concentration of potassium and a low concentration of sodium. The central compartment, the scala media, is roughly triangular in shape and is bounded by Reissner's membrane, basilar membrane, and stria vascularis. The organ of Corti rests on the basilar membrane. Scanning electron microscopy allows detailed three-dimentional views of the entire surface of the organ of Corti with its inner hair cells, outer hair cells, supporting cells (Deiter, Henson, Claudius, and pillar), the afferent and efferent nerve fibers, the tectorial membrane, and the basilar membrane (fig. 2).

The hair cells represent the mechanoreceptors responsible for sound transmission and, in mammals, are divided into one row of inner hair cells and three or more rows of outer hair cells. The inner hair cells are flask-shaped with relatively small nuclei whereas the outer hair cells are conical with nuclei that almost fill the diameter of the cell (fig. 2). The surface of the hair cells bears stereocilia which are arranged in the form of the letter "V" or "W" for the outer hair cells and are fencepost-like for the inner hair cells.

Figure 2

Three-dimensional schema of the structure of the organ of Corti. Demonstrated here is the arrangement of various types of cells and their overall innervation. The organization of the inner and outer hair cells, and their synaptic connexions are depicted below (Williams and Warwick, 1980).


Stria vascularis
Tectorial membrane
Inner hair cells
Outer hair cells
Spiral limbus
Hensen's stripe
Pillar cells
Reticular lamina
Habenula perforata
Tunnel of Corti
Basilar membrane
Spaces of Nuel
Cells of Hensen
Outer spiral sulcus
Cells of Claudius
Osseous spiral lamina
Inner spiral sulcus
Cuticular plate
Centriole
Stereocilia
Phalangeal process
Cell of Deiters
INNER HAIR CELL
OUTER HAIR CELL

Physiological Considerations of the Cochlea

Sensory Hair Cell Transduction

Sound energy is conducted through the external and middle ear into the inner ear where the transduction of mechanical stimuli into an electro-chemical form takes place. Several theories have been proposed to account for this transduction process. The most commonly accepted one, Davis' "Battery Theory", will be considered here.

Davis proposed that the upper surface of the hair cell of the cochlea is polarized by the difference between the intra-hair cell potential and the endocochlear potential generated by the stria vascularis. In response to sound stimulus, the up and down motion of the basilar membrane produces shearing action in the stereocilia embedded in the tectorial membrane. The resulting distortion in the membrane of the upper surface of the hair cell decreases the resistance of the membrane to current flow and in turn causes a depolarization of the hair cell. The depolarization of the hair cell activates the release of an unknown chemical transmitter and excitation of the VIII nerve.

Although Davis' theory appears to be currently the best working model for understanding sensory transduction in the cochlea, recent data suggest that a number of fundamental gaps with regard to this theory remain

unresolved. Lim ('72) demonstrated that outer hair cell stereocilia but not inner hair cell stereocilia are embedded in the tectorial membrane. These results, in conjuction with the Davis model, suggest that outer hair cells are the primary sensory receptor cell. Inconsistent with this proposal, Spoendlin ('72) demonstrated that inner hair cells receive 95% of the afferent innervation and have no direct efferent nerve endings, whereas, outer hair cells receive only 5% afferent nerve endings.

Outer hair cells receive a large number of efferent endings, many of which are from axons of the crossed olivocochlear bundle. Electrical stimulation of the efferent nerves in the olivocochlear bundle markedly reduced receptor potentials recorded from inner hair cells. (Brown et al., '83).

As previously mentioned, the primary source of mechanical input to cochlear hair cells is thought to be the relative shear between the tectorial membrane and the apical surface of the hair cells caused by basilar membrane motion during rarefactions (excitatory) and compressions (inhibitory) of acoustic stimuli (Davis, '65). Recent theories of cochlear transduction propose that outer hair cells, by virtue of the firm attachment of their stereocilia to the tectorial membrane (Lim,'72), are in a position to regulate this displacement through a

bidirectional mechano-electrical feedback loop (Brownell et al. '85; Cody and Russell, '85). Brownell et al. ('85) supported this theory with observations that isolated outer hair cells became shorter in response to depolarizing currents and longer in response to hyperpolarizing currents administered to the synaptic end of the cell. Iontophoretic application of acetylcholine to the synaptic end of the cell decreased the length of the outer hair cell.

It is not known at this time exactly how the inner hair cell stereocilia are mechanically distorted in response to sound stimuli. Russell and Sellick ('83) suggested that the inner hair cells respond to the shear of their free-standing stereocilia through a fluid coupling to basilar membrane action.

Compound Action Potential.

An electrode on the round window or in the cochlea may record a transient response with onset of acoustical stimulation. This response is the cochlear compound action potential and represents the discharge of afferent fibers of the VIII nerve. It is usually characterized by two deflections, an N_1 peak and an N_2 peak. Each peak is separated by approximately 1-1.5 msec. The action potential is highly dependent on the synchrony of discharge of the auditory nerve fibers and on the number of nerve

fibers excited (Goldstein et al., '58). Optimum response is initiated with sounds of short duration such as a click or tone burst. The compound action potential is an indicator of cochlear end function and is an important measure for determining changes in hearing as a result of noise exposure, ototoxic drugs, or other insults to the cochlea (Aran, '81).

Cisplatin

Pharmacology

The structure of cisplatin consists of a platinum moiety surrounded by two chloride groups and two amino groups. Cisplatin has a molecular weight of 300 and is soluble in water at a concentration of 1 mg/ml (Dorr and Fritz, '80). The reactivity of this drug in biological systems is dependent on chloride ion concentration. In an environment of high chloride concentration (approximately 100 mM) as in blood, cisplatin remains electrically neutral (Lippard, '80). Inside the cell where the chloride ion concentration is approximately 4mM, the chloride-leaving groups are replaced by water molecules resulting in a positively charged, aquated platinum molecule (Lippard, '80). The positively charged molecules may react with nucleophilic sites within the cell. Amine ligands are strongly bound to the central metal atom and are unlikely

to participate in binding. The principle target of cisplatin appears to be DNA, with a selective inhibition of DNA synthesis (Harder and Rosenberg, '70). The $t_{1/2}$ of the drug in rodents with i.v. administration is 25-50 minute for the initial distribution phase and 58 to 73 hours for the elimination phase (Hill et al., '75; Calabresi and Parks, '80). Distribution occurs in high levels to kidney, liver, ovary, uterus, intestine, skin, and bone; and occurs in low levels to the central nervous system (Litterst et al., '79). Ninety percent of cisplatin administered is bound to plasma proteins and 70-90% is excreted by the kidneys. In addition to ototoxicity, the major side effects of this agent include nephrotoxicity, myelo-supression, and gastrointestinal disturbances (Hoff et al., '79).

Ototoxicity

Cisplatin-induced hearing impairment has been reported in a number of clinical studies (Piel et al., '74; Merrin, '78; Calabresi and Parks, '80). Reported hearing losses were dose-dependent, usually bilateral, and were both reversible and irreversible. Hearing impairment occurred at 4000-8000 Hz and ranges between 15-65 dB (Moroso and Blair, '83).

Results from animal studies indicated that cisplatin destroyed outer hair cells of the organ of Corti with the

greatest damage occurring in the basal turn (Nakai et al., '82; Tange et al., '82; Fleishman et al., '75). Occasional destruction of inner hair cells has been observed but was consistently less than for outer hair cells and occurred only at the higher doses.

Ultrastructural changes associated with outer cell degeneration, as visualized with transmission electron microscopy, included marked vacuolization and large numbers of lysosome-like bodies in the apical region of the cell, thinning of cuticular plate, and enlargement of the parietal membrane (Estrem et al., '81). In stria vascularis, occasional vacuole enlargement and thinning of the membrane of cells was reported (Nakai et al., '82). Nakai et al. ('82) observed larged fused stereocilia, distorted and distended cuticular plates, and pronounced hair cell loss using scanning electron microscopy.

In addition to morphological changes induced by cisplatin, functional changes have also been reported. Hearing function in guinea pigs after multiple doses of cisplatin, i.p. (total of 15 mg/kg over 12 days), resulted in permanent deafness with hearing loss for high frequency tones preceeding that of low frequencies (Fleischman et al., '75). In guinea pigs treated with 2 mg/kg/day cisplatin (i.m.), Nakai et al. ('82) reported complete suppression of the auditory brainstem response. Komune

et al. ('81) observed significant reduction of both the endocochlear potential and cochlear microphonics following a single i.v. injection of 12.5 mg/kg cisplatin in guinea pigs.

The morphological and functional changes produced by cisplatin are probably an end result, reflecting biochemical or biophysical alterations taking place in various structures of the cochlea (Konishi et al., '83). Although these published reports clearly demonstrated the dramatic ototoxic effects of cisplatin, none of these data identified possible mechanisms underlying cisplatin-induced cochlear damage.

Recently, Daley-Yates and McBrien ('82) reported a dose-dependent inhibition of Na,K-ATPase and Mg-ATPase activity in renal tissue incubated with cisplatin (ID_{50} 6.5 x 10^{-4}M). Although these results are not direct evidence for a mechanism of cisplatin ototoxicity, similarities between the kidney and the cochlea should not be overlooked. Good correlations exist between the ototoxicity produced by certain drugs and their renal toxicity (Brown and Feldman, '78). Antigenic similarity between the cochlear stria vascularis and the glomerulus, distal convoluted tubules, and the collecting ducts of the kidney have been reported by Quick et al. ('73). Futhermore, the kidney and the cochlea are each composed of

highly specialized tissues whose function in part is the transport of water and electrolytes across cellular barriers (Brown and Feldman, '78).

The active transport of sodium and potassium across cellular membranes is of great physiological significance and is known to be catalysed by the enzyme Na,K-ATPase. Iinuma ('69) and Bonding ('59) determined biochemically the presence of high Na,K-ATPase activity in stria vascularis but low activity in the remaining cochlear tissues. Ultrastructural localization of Na,K-ATPase activity within the guinea pig cochlea demonstrated the greatest concentration to be in stria vascularis, sparser concentration in spiral prominence, and no activity in the remaining cochlear tissue (Mees, '83).

Na,K-ATPase in the stria vascularis is thought to be largely responsible for maintaining the distinct ion composition of the endolymph (high potassium and low sodium). Alteration of endolymphatic ionic composition has been demonstrated to produce deterioration in the maintenance of normal electrical activity within the cochlea (Brown, '75) and structural damage to the hair cells (Kronester-Frei, '79).

Objectives

It can be postulated from the findings of previous studies that the primary mechanism of cisplatin ototoxicity

may involve the enzyme, Na,K-ATPase.

The purpose of this investigation was to determine the effect of varying doses of cisplatin on Na,K-ATPase activity in the lateral wall (stria vascularis and spiral ligament) and to correlate this data with cisplatin-induced functional and morphological changes in the cochlea. Functional changes were assessed with measurements of loss of Preyer reflex and recordings of compound action potential. Morphological changes were visualized with scanning electron microscopy.

Null Hyothesis

There is no correlation between cisplatin ototoxicity and Na,K-ATPase activity in the lateral wall of the cochlea.

CHAPTER 2

Methods and Materials

Test Animals and Treatment Schedule

Female Hartley albino guinea pigs, weighing from 300-400g were utilized in 4 treatment groups and 1 control group. The guinea pigs had free access to Wayne rabbit ration and water containing ascorbic acid. They were maintained on a light-dark cycle (12L:12D) with the dark cycle occurring between 1900-0700 hours. The temperature of the animal room was 22 +/- 1°C. The time of sacrifice was between 0900 and 1400 hours. The guinea pigs and treatment levels were randomly selected by assigning numbers to the guinea pig and treatment level, placing the numbers in a container, and drawing from the container the number of the animal and treatment to be used. Treatment regimens consisted of daily intraperitoneal (i.p.) injections of either cisplatin (Sigma) or sterile water for 12 consecutive days prior to sacrifice:

Group	Cisplatin (mg/kg per day)	Total cisplatin dose (mg/kg)
1	0.00	0.00
2	0.42	5.00
3	0.83	10.00
4	1.17	14.00
5	1.50	18.00

Cisplatin solutions were prepared daily by diluting 10 mg cisplatin in 10 ml sterile water. Measurements of the various parameters were performed on day 13.

Electrophysiological Method for Recording Compound Action Potential from the Round Window

Guinea pigs were screened for normal hearing function with a Preyer reflex test each day of treatment and on the day of testing. A positive Preyer reflex was represented by the cocking of the guinea pig pinnae in response to a finger click. The guinea pigs were anesthetized with 2 mg/kg Urethane in 0.9% saline, i.p., a tracheotomy was performed, and the body temperature was maintained with a heating pad. A modification of the method of Daigneault et al. ('68) was used to record the compound action potential from the round window. The bulla was exposed through a submandibular approach. The tympanic membrane, bony cochlea, round window, and oval window were exposed by first drilling a 1 mm diameter hole in the bulla (dental drill and burr) and then further enlarging the opening with forceps. A Formvar-coated nickel wire electrode (dia. 0.5 mm) was placed on the bony ridge immediately above the round window. The indifferent electrode was placed in one of the neck muscles. The shields of the active and indifferent electrodes were connected to the ground electrode. The signal was fed into a Grass model P15

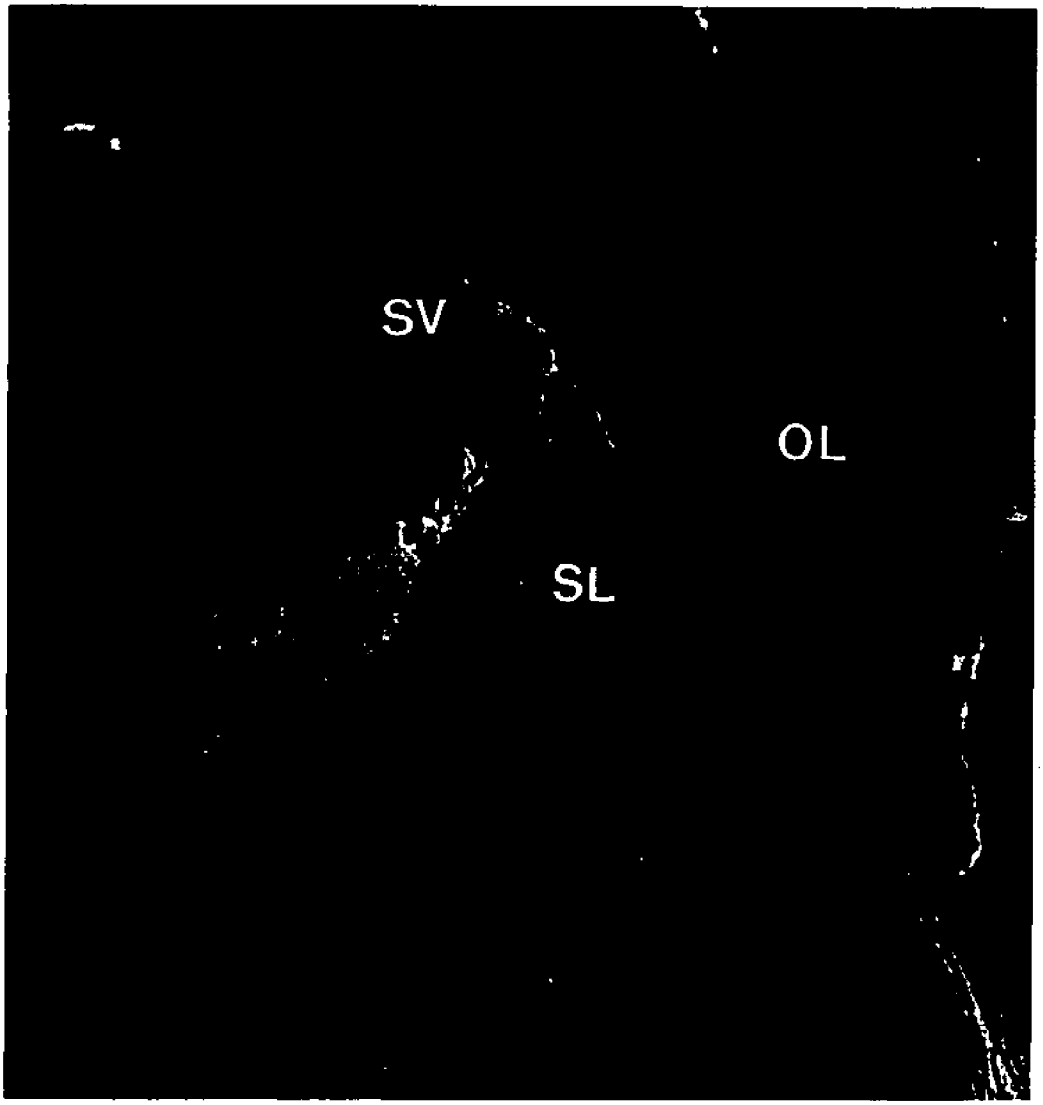
differential A/C microelectrode preamplifier. A click stimulus of short duration was generated in the TDH39 Telephonics earphone by a square wave (0.02 msec duration, 1 volt) placed across the terminals and produced by a Grass S88 stimulator. The tip of a small speculum fitted in the earphone was placed just inside the auditory canal of the guinea pig and was immobilized. The intensity of the sound stimulus at the tip of the speculum was approximately 60dB as measured by a Bruel and Kjaer sound level detector. The output was fed into a Nicolet (model NIC MED-80) Data Acquisition System for signal averaging and data storage (20 sweeps; 10 msec sweep time). The data was expressed as changes in amplitude (μV) and latency (msec). Latency was defined as the time between initiation of click and peak of N_1.

Determination of Na,K-ATPase Activity

After completion of the recording of the compound action potential from the round window, the animal was decapitated and the petrous bone from each temporal bone was removed from the skull. The cochlea to be used for determination of enzyme activity was placed immediately in ice-cold distilled water. Under a dissecting microscope, the thin osseous labyrinth of the cochlea was picked away to expose the spiral ligament. Figure 3 demonstrates the anatomical relationship between the lateral wall (spiral

Figure 3

Micrograph demonstrating the anatomical relationship of the lateral wall with the osseous labyrinth (OL). The lateral wall consists of the stria vascularis (SV) and the spiral ligament (SL). Bar indicates 50 µm.


SV
OL
SL

ligament and stria vascularis) and the osseous labyrinth. In unfixed tissue, as utilized for determination of Na,K-ATPase activity, the lateral wall is in intimate contact with the osseous labyrinth. Precise dissection of the stria vascularis from the spiral ligament was not consistently achieved by this investigator. Therefore, specimens of the entire lateral wall were pulled away from the basilar membrane and placed in 5 µl ice-cold distilled water. The tissue was hand-homogenized with a tube-and-pestle tissue grinder (100-1000 µl capacity; Fisher) and additional distilled water (approximately 20 µl) was added to wash the sides of the tube. The tissue was lyophilized at -70°C overnight and stored at -20°C. After reconstituting the freeze-dried tissue with 125 µl distilled water, 10 µl of the enzyme suspension was added to 100 µl of reaction medium; (60 mM NaCl, 6 mM KCl, 3 mM $MgCl_2$, 30 mM Tris, 3 mM Adenosine 5'-Triphosphate [disodium salt, equine muscle], pH 7.3) and incubated at 37 °C with agitation for 1 hour. The linearity of the reaction with a one hour incubation time and a protein concentration of 1-5 µg was previously determined by Iinuma ('67) and confirmed in preliminary experiments by this investigator. To measure Na,K-ATPase activity resistant to ouabain (Mg-dependent ATPase), 10^{-4} M ouabain was included in the preincubation mixture. The reaction was stopped with

100 µl of ice-cold 10% w/v trichloroacetic acid. A modification of the method of Fiske and SubbaRow ('25) was used for phosphate determination; 75 µl of distilled water, 100 µl of molybdate-H_2SO_3 in 15% $NaHSO_3$ and 25 µl reducing reagent (1,2,4-aminonapthalsulfonic acid in 15% $NaHSO_3$) was added to the reaction mixture. The concentration of protein in the aliquots of homogenate was measured using the method of Lowry et al. ('51). Measurements of Na,K-ATPase activity were calculated as the difference between the activities obtained from ouabain-absent and ouabain-present media and were expressed as micromoles of inorganic phosphate split off from ATP per mg protein per hour (µmoles Pi/mg protein/hr).

Scanning Electron Microscopy

The dissected petrous bone with cochlea intact was immersed in fixative consisting of 4% glutaraldehyde and 4% sucrose in 0.2M s-collidine buffer, pH 7.3. While immersed in fixative, the stapes was removed from the oval window and the round window was punctured with a micro-probe. A hole was placed in the apex with a micro-probe. The fixative was gently and slowly perfused through the round window using a syringe with tubing attached to a 22 gauge needle. The cochlea remained in fixative for 24 h. Decalcification was carried out in 5% EDTA (disodium salt) in 0.1M phosphate buffer. The solution was changed once

after 24 h and the cochlea remained in solution for an additional 24 h. The cochlea was washed with 4% glutaraldehyde in 0.1M phosphate buffer, pH 7.3. The tissue was post-fixed for 1 h in cold 2% OsO_4 (Polysciences, Inc.) in 0.1M phosphate buffer, pH 7.3 followed by a wash with phosphate buffer (0.1M, pH 7.3). Next the cochlea was dehydrated through a graded series of ethanol/H_2O solutions (30%, 50%, 70%, 80%, 90%, 95%, and 100%) with microdissection completed at 70% H_2O/ethanol. The tissue was critical point dried with CO_2 as the transitional fluid, mounted on an aluminum stub with silver paste, and coated with gold-palladium in a sputter-coater. The specimen was examined with an Hitachi S430 scanning electron microscope with special attention given to the morphology of the inner and outer hair cells. Measurements of hair cell loss were determined by counting the number of missing hair cells out of a segment of the basilar membrane normally containing 50 hair cells per row.

Statistics

The mean and the standard error of the mean were determined for experiments measuring hair cell loss, Na,K-ATPase activity and N_1 amplitude. One-way analysis of variance (ANOVA) was used for the statistical evaluation and the Duncan test was used for post hoc comparison.

CHAPTER 3

Results

Preyer Reflex Test

Cisplatin-induced hearing loss as determined by Preyer reflex test was dose-related (Table 1). A positive Preyer reflex occurred in guinea pigs from the control group and from the 0.42 dose group. In the 0.83 and 1.17 mg/kg dose groups, 2/6 and 4/6 guinea pigs exhibited a loss of Preyer reflex response, respectively. All of the guinea pigs in the high dose group (1.5 mg/kg) showed a lack of Preyer reflex response. The average number of days between the first day of treatment and loss of Preyer reflex appearred to be inversely dose-related.

The majority of the guinea pigs treated with varying doses of cisplatin were active, exhibited normal grooming habits, and displayed a normal gait. Only one case of mortality occurred and this was a 1.5 mg/kg dose treated animal (Table 1).

Compound Action Potential

The amplitude of the N_1 response to an one volt click was -91.00 +/- -4.30 μV and the latency was 1.47 +/- 0.04 msec. In cisplatin-treated animals, suppression of N_1 amplitude and increase in latency appeared to be dose-related but varied a great deal between animals of the same treatment group (Table 2). The amplitude and latency of N_1

TABLE 1

PREYER REFLEX TEST IN GUINEA PIGS

AFTER 12 DAYS OF CONSECUTIVE CISPLATIN TREATMENT

Daily Dose (mg/kg)	Number That Died/ Number Treated	Number with Preyer Reflex Loss/Number Tested	Average Day of Loss of Preyer's Reflex
0.00	0/6	0/6	-
0.42	0/6	0/6	-
0.83	0/6	2/6	11
1.17	0/6	4/6	10
1.50	1/6	6/6	8

TABLE 2

CISPLATIN'S EFFECT ON COMPOUND ACTION POTENTIAL (CAP) IN GUINEA PIGS

Dose (mg/kg)	N_1 of CAP[a]	
	Amplitude (μV)	Latency (msec)
Control	-91.0 +/- -4.3	1.47 +/- 0.04
0.42	-71.2 +/- -17.7	1.63 +/- 0.06
0.83	-48.2 +/- -11.1	1.66 +/- 0.05
1.17	-49.2 +/- -22.8	---[b]
1.50	---[c]	---[c]

[a] Values represent mean +/- SEM, n=6.

[b] Absence of response (n=3) prohibited calculation of mean.

[c] Complete suppression of CAP in all guinea pigs.

was not statistically different between treatment groups at $p<0.05$. Complete suppression of the compound action potential occurred in 50% of the 1.17 mg/kg and all of the animals in the 1.50 mg/kg dose groups. A good correlation existed between suppression of the action potential and loss of Preyer reflex. Representative tracings of the action potential are shown in figure 4. The first pronounced peak seen is classified as N_1. Values for N_1 in figure 4 are $^{-}94$ μV, 1.56 msec for the control and $^{-}56$ μV, 1.79 msec for the 0.83 mg/kg treated animal. Complete suppression of the action potential occurred in the guinea pigs treated with 1.50 mg/kg cisplatin.

Scanning Electron Microscopy

Scanning electron microscopy revealed surface morphology of many cochlear structures including the inner hair cells, outer hair cells, supporting cells, Reissner's membrane, tectorial membrane and the modiolus (fig. 5a). The orderly pattern of the 3 rows of outer hair cells and one row of inner hair cells is obvious in figure 5b in addition to the "V" or "W" shape of the individual outer hair cell stereocilia and fencepost-shape of the inner hair cell stereocilia.

In guinea pigs treated with cisplatin, damage occurred sporadically throughout the turns of the cochlea with greatest predominance in the basal turn. The percentage of

Figure 4

Representative tracings of the cochlear compound action potential initiated with an one volt click (arrow). The first pronounced negative peak is classified as N_1.

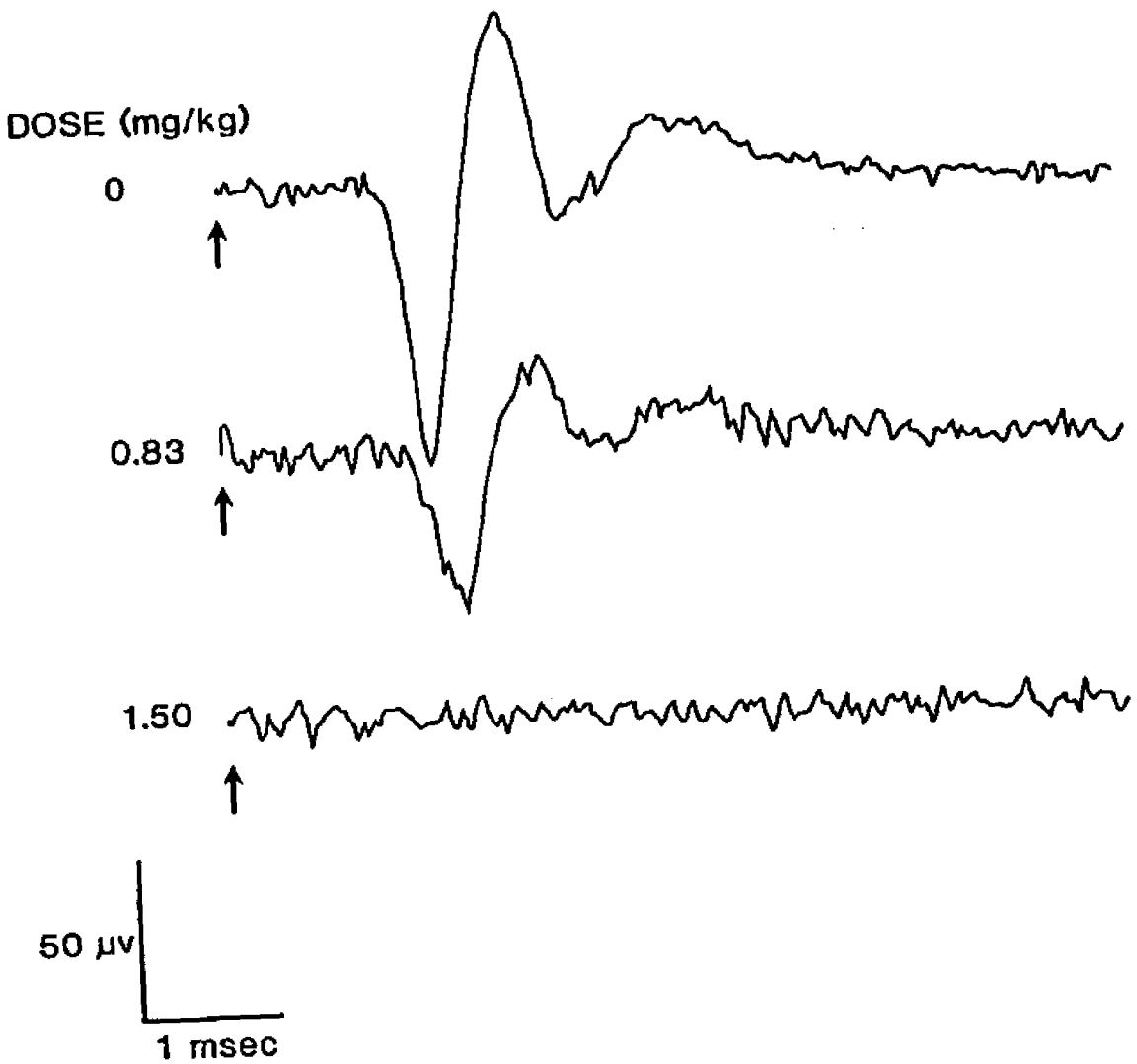
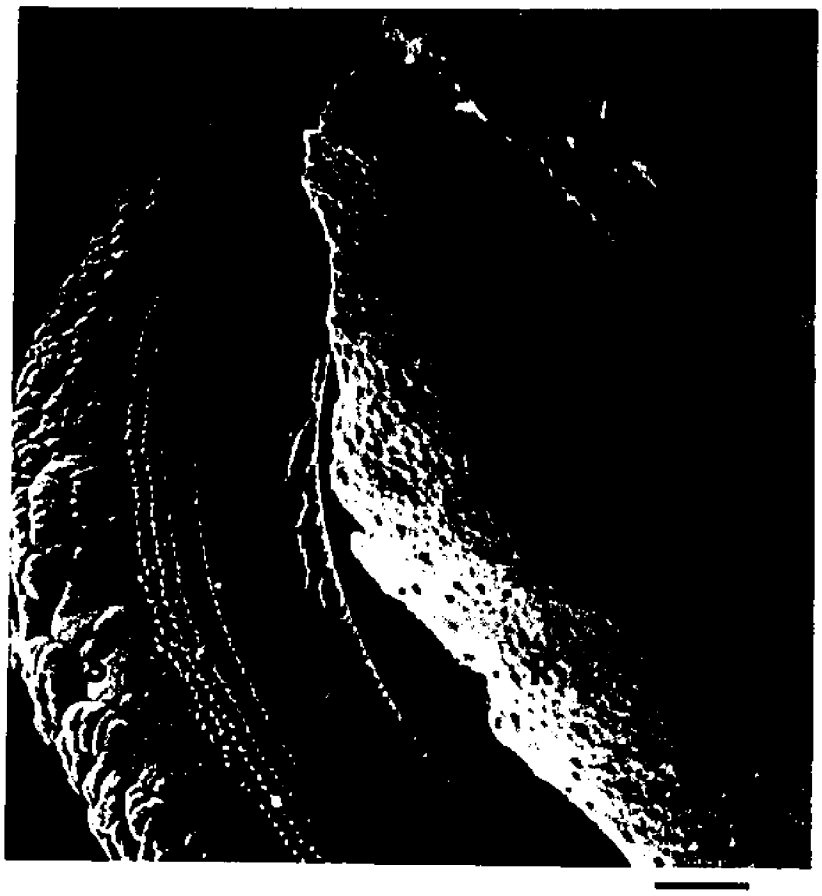

DOSE (mg/kg)
0
0.83
1.50
50 µv
1 msec

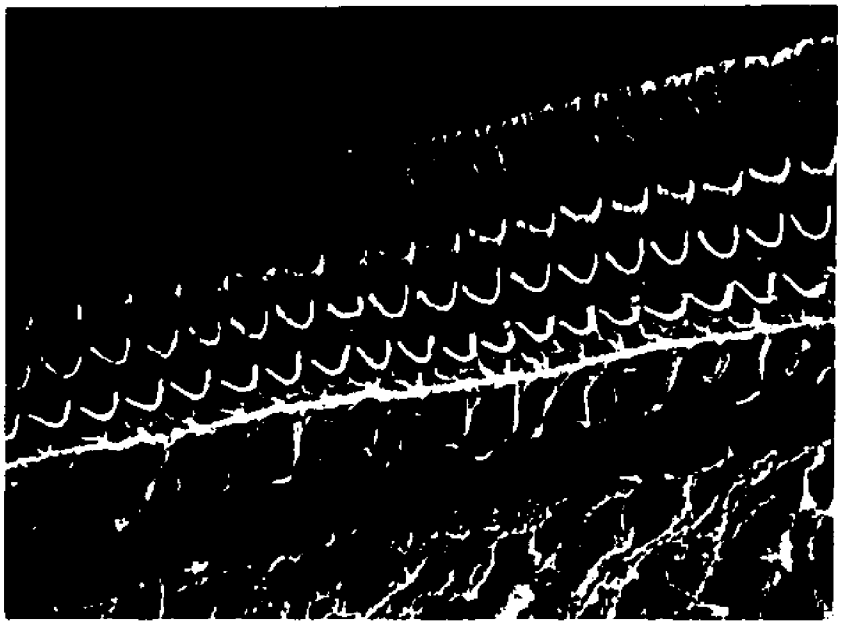
Figure 5

Visualization with scanning electron microscopy of cochlear structures in non-treated guinea pigs. 5a. View of a portion of one of the four and one half turns of the cochlea. Shown here are Reissner's membrane (R), tectorial membrane (T), inner hair cells (I), outer hair cells (O), the supporting cells (S), and the modiolus (M). Bar indicates 50 µm. 5b. Higher magnification of the hair cells. Note the orderly and characteristic pattern of the stereocilia. Bar indicates 5 µm.

5a



5b



missing hair cells out of a total of 50 hair cells in the basal turn (approximately 2mm from round window) was calculated for the various concentrations of cisplatin administered (fig. 6). Micrographs representing cisplatin-induced damage at different dosages administered are shown in figure 7 and 8. The basal turn proved to be the most reliable area for quantitation since a greater incidence of anatomical variation occurred in the upper cochlear turns. The inner hair cells were much more resistant to loss of stereocilia than outer hair cells at these dose levels. Mean values of the percentage of missing cells for outer hair cell rows one, two, and three were not significantly different from each other. At 0.42 and 0.83 mg/kg dose levels, the number of outer hair cells missing was not statistically different from control animals although a tendency for an increase in loss was seen at 0.83 mg/kg cisplatin. A dramatic loss of outer hair cells occurred at 1.17 and 1.5 mg/kg cisplatin.

Changes in hair cell morphology, in addition to complete loss of hair cell stereocilia, occurred throughout all turns of the cochlea and in both inner hair cells and outer hair cells. Degrees of hair cell stereocilia distortion were represented by separation, spraying, clumping, formation of blebs on the plasma membrane, expulsion of hair cell contents into endolymphatic space,

Figure 6

Dose-response curve of the percentage of hair cell loss in the basal turn. Missing hair cells were determined from a total of 50 hair cells in each row; inner hair cells (IHC), outer hair cell row one (OHC1), outer hair cell row two (OHC 2), and outer hair cell row three (OHC3). Values represent mean and bars represent SEM (n=6).

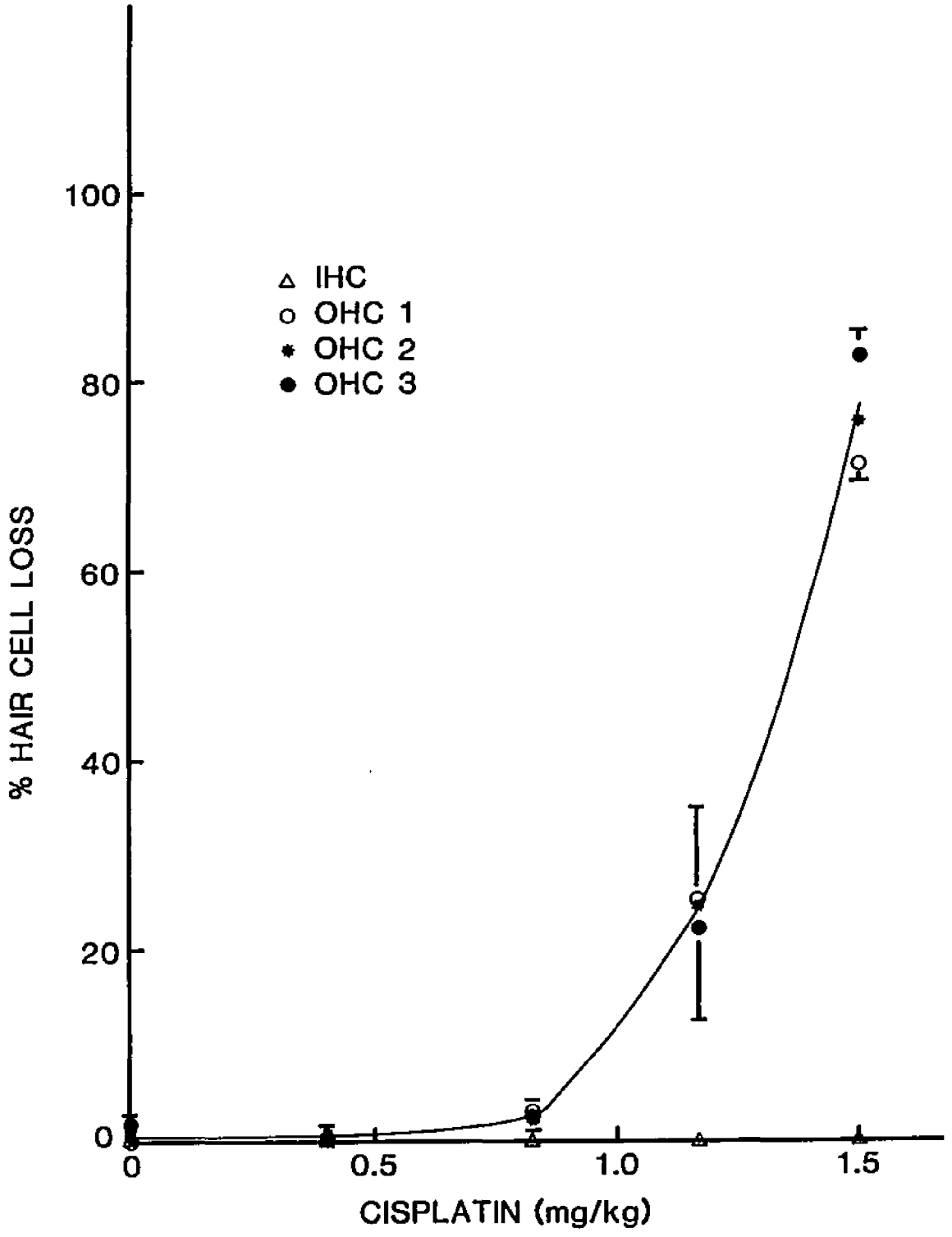

100
80
60
40
20
0
% HAIR CELL LOSS
△ IHC
○ OHC 1
✱ OHC 2
● OHC 3
0
0.5
1.0
1.5
CISPLATIN (mg/kg)

Figure 7

Visualization of hair cell stereocilia damage at different cisplatin treatment levels. 7a. 0.42 mg/kg 7b. 0.83 mg/kg. Bars indicate 5 μm.

7a

7b

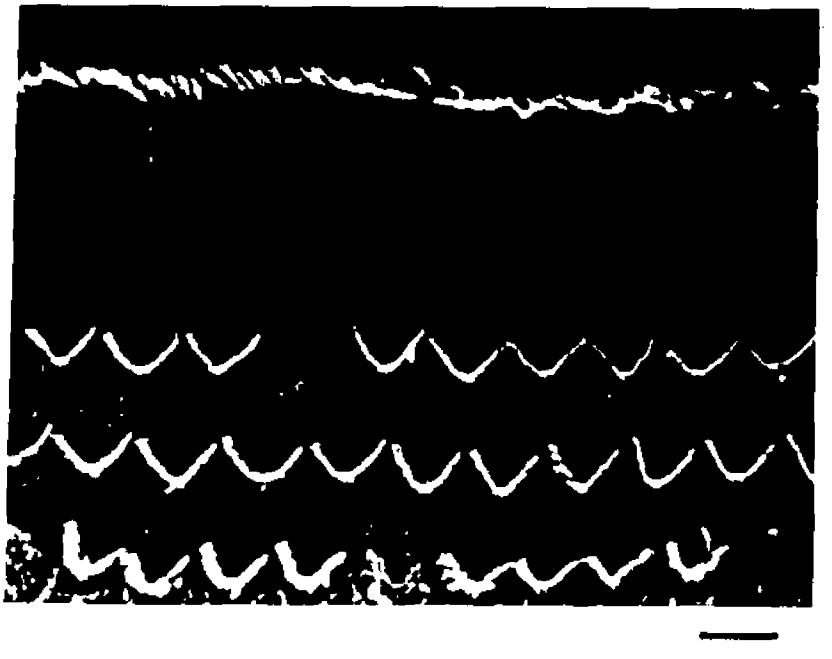


Figure 8

Visualization of hair cell stereocilia from cisplatin-treated animals. 8a. 1.17 mg/kg 8b. 1.50 mg/kg. Bars indicate 5 µm.

8a

8b

and scarring of the phalangeal plate (Fig. 9 and 10). Of these degrees of hair cell distortion, only separation and spraying of stereocilia and scarring of the phalangeal plate were significantly observed in the 0.42 and 0.83 dose groups. All degrees of hair cell distortion and the presence of cellular debris were noted in the higher doses (1.17 and 1.50 mg/kg cisplatin).

Surface morphology of other cochlear structures such as stria vascularis, supporting cells, tectorial membrane, and Reissner's membrane were examined in addition to the hair cells but detectable morphological changes induced by cisplatin treatment were not observed.

Na,K-ATPase Activity

The mean level of Na,K-ATPase activity in the cochlear lateral wall of control guinea pigs was $1.21^{+}/-0.23$ µmol Pi/mg protein/hr. Na, K-ATPase activity was not statistically significant between treatment groups. Mean values $^{+}/-$ S.E.M. of Na,K-ATPase activity for each treatment groups are shown in figure 11.

Figure 9

Scanning electron micrograph demonstrating various degrees of hair cell stereocilia distortion. 9a. Separation and spraying in outer hair cells. 9b. Formation of blebs in inner hair cells. Bars indicate 5 μm.

9a

9b

Figure 10

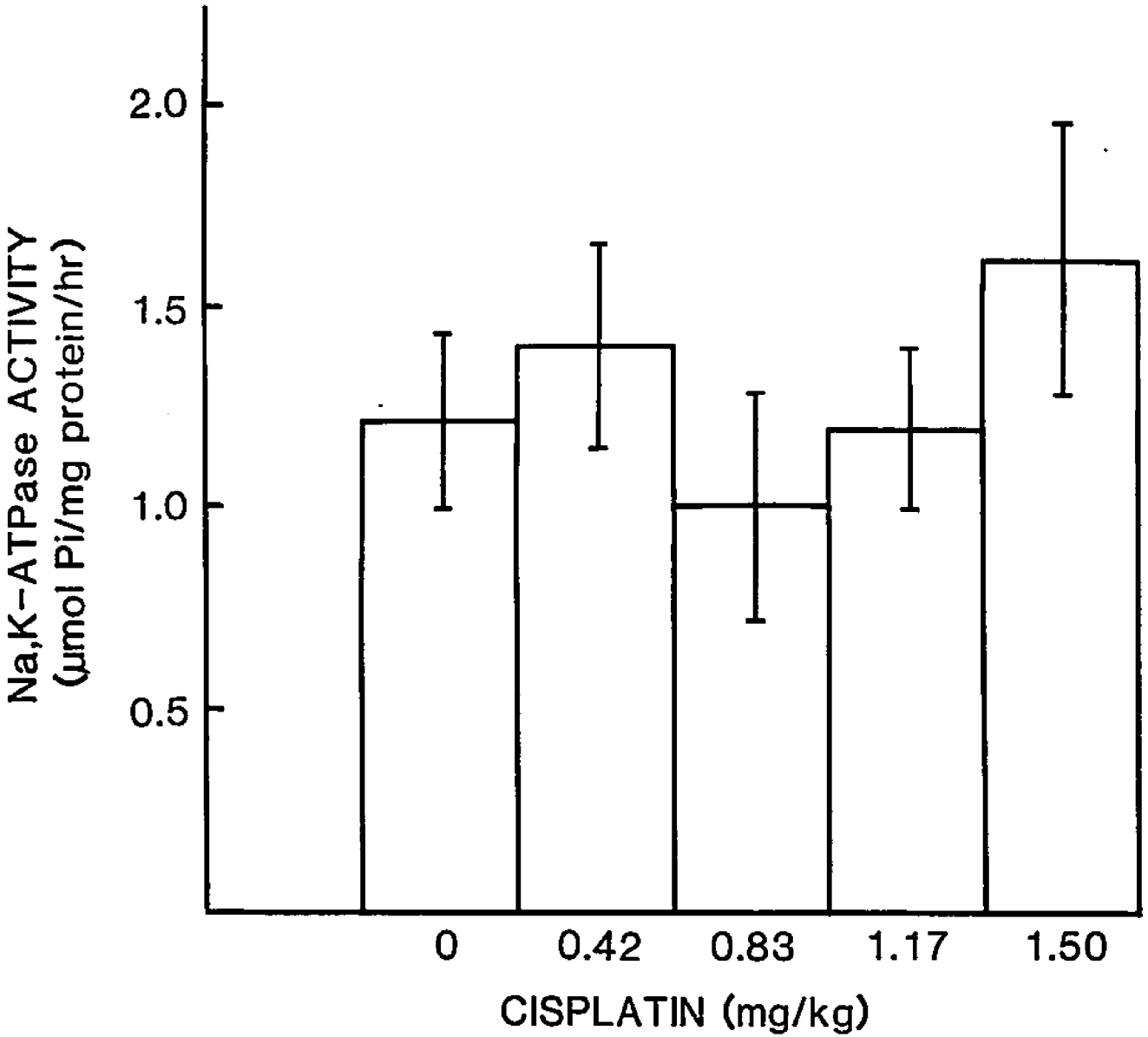
Scanning electron micrograph demonstrating hair cell stereocilia distortion. 10a. Clumping in inner hair cells. 10b. Expulsion of outer hair cell contents into endolymphatic space. Bars indicate 5 μm.

10 a

10 b

Figure 11

Na,K-ATPase activity (μmol Pi/mg protein/hr) in the lateral wall of the cochlea after treatment with cisplatin or sterile water. Values shown are the mean +/- SEM, (n=6).


Na,K-ATPase ACTIVITY
(μmol Pi/mg protein/hr)
2.0
1.5
1.0
0.5
0
0.42
0.83
1.17
1.50
CISPLATIN (mg/kg)

CHAPTER 4

Discussion

Introduction

The objectives of this investigation were twofold. First, the effects of varying doses of cisplatin were to be examined with regard to functional changes as determined by measurements of Preyer reflex loss and cochlear compound action potential and with regard to morphological changes as visualized with scanning electron microscopy. Secondly, the presence or absence of a correlation between cisplatin ototoxicity and Na,K-ATPase in the lateral wall was to be determined. These goals were ultimately achieved.

Dose Effects of Cisplatin

Guinea pigs treated for 12 consecutive days with a graded dose range from 0.42 to 1.50 mg/kg cisplatin, i.p., demonstrated dose-dependent ototoxic effects. Hearing function, as determined with recordings of compound action potential and with Preyer reflex test, ranged between normal values to complete hearing impairment. Deafness, as determined by loss of Preyer reflex, occured at a total cisplatin dose between 9 and 12 mg/kg in all treatment groups. Observations from scanning electron microscopy indicated no significant morphological changes at low doses, but did indicate dramatic hair cell lesions at high

doses. Only one case of mortality occurred for the doses administered and this was a 1.5 mg/kg treated animal. In agreement with these findings, Fleischman et al. ('75) reported deafness (loss of Preyer reflex) in all guinea pigs treated with 1.50 mg/kg cisplatin, i.p., five times per week for an average of 12 days and established the LD_{50} to be greater than 1.5 mg/kg. The incidence of mortality in the present study and that of Fleischman et al. ('75) may be attributable to the nephrotoxic actions of cisplatin. Nephrotoxicity is the dose-limiting factor for cisplatin treatment and is potentially life-threatening (Goldstein and Mayor, '83).

Na,K-ATPase Activity in Cochlear Lateral Wall

Cisplatin was suggested, by this author, to cause cochlear toxicity by altering the level of Na,K-ATPase activity in the lateral wall, thereby causing abnormal functioning of the cochlea. Our data, however, demonstrated that the level of Na,K-ATPase activity in the lateral wall was not significantly different between the control group and the cisplatin-treated groups. Cisplatin ototoxicity, as determined by measurements of Preyer reflex, compound action potential and morphological changes in hair cells, was not directly related to Na,K-ATPase activity in the lateral wall. The null hypothesis in the present study, therefore, was not rejected. These results

support recent findings of Konishi et al. ('84), who treated guinea pigs with 1.5 mg/kg cisplatin, i.p., five times a week until electrocochleograms showed persistent and complete supression of the action potential. Results from the electrophysiological and biochemical measurements showed little or no effect of cisplatin treatment on the endocochlear potential or on the electrolyte concentrations in endolymph and perilymph. The findings of our experiments and those of Konishi et al., ('84) suggest that the primary ototoxic effect of cisplatin is not on the enzyme, Na,K-ATPase in the lateral wall.

Morphological Changes in Sensory Hair Cells

Possible sites for the toxic actions of a drug in the cochlea include; inner hair cells, outer hair cells, supporting cells, stria vascularis, Reissner's membrane, and the tectorial membrane. Results from observations with scanning electron microscopy in the present study suggest that cisplatin's primary toxic effect was on the outer and inner hair cells.

The pattern of hair cell degeneration induced by cisplatin was determined. Sporadic degeneration of the hair cells occurred throughout the organ of Corti with a strong prevalance in the basal turn and with no significant difference between rows of outer hair cells. Outer hair cell loss appeared to be dose-dependent. Inner hair cells

were much more resistant to drug-induced loss of stereocilia. In contrast to these results, Konishi et al. ('83), Nakai et al. ('82), Tange et al. ('80), and Fleischman et al. ('75) reported that outer hair cells row one was more susceptible to damage than outer hair cells rows two and three. Upon close examination of published cochleograms of the basal turn from Tange et al. ('82) and from Fleischman et al. ('75), the rows of outer hair cells did not appear to be significantly different. Cochleograms or actual data for hair cell loss was not published in reports by Konishi et al. ('83) or by Nakai et al. ('82).

The degeneration pattern of cisplatin appears to be somewhat different from the aminoglycosides. Tange et al. ('82) reported that the degeneration process for cisplatin intoxicated outer hair cells is more sporadic while the gentamicin degeneration is more continuous. Our data is in agreement with Tange showing sporadic hair cell degeneration throughout the turns of the cochlea.

It is not fully understood at this time why outer hair cells are more vulnerable than inner hair cells. It has been suggested by Lim ('71) that outer hair cells are metabollically more active than inner hair cells and, therefore, more susceptible to trauma. In addition, outer hair cells, unlike inner hair cells are surrounded by large extracellular spaces (spaces of Nuel, fig. 1) that may

allow greater exposure to drugs possibly localized in the cortilymph.

Distortion of hair cell stereocilia occurred in both inner hair cells and outer hair cells and was represented by separation, spraying, formation of blebs, clumping, expulsion of hair cell contents, and scarring of the phalangeal plate. Hair cell stereocilia distortion has also been reported in aminoglycoside-intoxicated cochleas (Wersall et al., '73), in acoustic trauma (Lim, '76), and in the Waltzing guinea pig (Wit and Nijdam, '84). It was observed in the present study that while all degrees of hair cell distortion were found in the higher doses, blebing, clumping, expulsion of hair cell contents and the presence of cellular debris was not significantly seen in the 0.42 and 0.83 mg/kg dose groups. These results suggest that mechanisms within the cochlea are capable of clearing the cellular debris when the damage occurs at a slow rate and lower incidence. However, at the higher doses, these mechanisms may be over-loaded and the cellular debris from degenerating hair cells is accumulated. Lim ('76) reported that cell debris was phagocytized by macrophages in kanamycin-intoxicated cochleas.

Cochlear Compound Action Potential

In the present study, recordings of the cochlear compound action potential were sensitive to functional

changes induced by cisplatin and appeared to be dose-dependent. Konishi et al. ('83) and Nakai et al. ('82) reported similar suppression of N_1 amplitude measurements of auditory brainstem response.

The amplitude and latency of the N_1 response is a function of both the stimulus intensity and the number of nerve fibers firing in synchrony. Since the intensity of stimulation remained constant in all experiments of the present study, the suppression of N_1 must be related to the number of nerve fibers firing and the synchrony of firing. The afferent activity in the primary auditory fibers is dependent on the excitation of the sensory cells. The results in the present experiment and that of Konishi et al. ('83) and Nakai et al. ('82) showed that guinea pigs exhibiting severe suppression of N_1 also showed dramatic loss of outer hair cells but preservation of inner hair cells. Although the relationship between outer hair cell loss and suppression of N_1 amplitude suggests that the outer hair cells are the primary auditory sensory cells, it has been well established by Spoendlin ('72) that inner hair cells receive the majority of afferent innervation. Two possible alternatives can therefore be suggested from the combination of previous data and those presented here. First, although the inner hair cells are anatomically intact, these cells may not be functional. Secondly, the

inner hair cells may be dependent on the outer hair cells for the normal transduction process to occur. It is the current thinking by many investigators that inner hair cells are the primary sensory receptor cells and that the mechanical input to the inner hair cells and thus their excitation are regulated by the outer hair cells, (Brownell et al., '85).

In the present study a large variation of the suppression of N_1 occurred between guinea pigs within the same treatment groups for doses less than 1.5 mg/kg. Variations in susceptibility to hearing loss have been reported by Konishi et al. ('83) in cisplatin treated animals and by Cody and Robertson ('83) in noise-induced trauma. Recently, Cody and Robertson ('83) investigated experimental factors responsible for this variation in suceptibility to hearing loss. Even with strictly controlled experimental procedures (pure tone stimuli in closed field, immobilized animals, careful measurement of the sound pressure at the tympanic membrane, and strict control of the sound pressure during exposure, by means of feedback control), Cody and Robertson reported large within group variations for both functional impairment and structural damage to hair cell stereocilia and concluded that unknown factors within the cochlea must be responsible for these variations.

Preyer Reflex Test

The Preyer reflex text allowed non-invasive screening of hearing function in the unanesthetized animal. Our results indicated that hearing loss as determined by loss of Preyer reflex was dose-dependent and occurred at a total cisplatin concentration between 9 and 12 mg/kg. In agreement with these results, Nakai et al. ('82) and Fleischman et al. ('75) reported hearing loss at total cisplatin concentration of 10 and 15 mg/kg respectively. The somewhat higher drug concentration used by Fleischman et al. may be due to a two day drug rest period per week during their treatment schedule.

Implications for Future Research

On the basis of the present findings, further investigation concerning the primary site of toxic action is needed before the mechanism of cisplatin's ototoxic actions can be fully understood.

Cisplatin's effect on hair cells such as the formation of blebs on the stereocilia and the expulsion of hair cell contents into the endolymphatic space suggest that this agent may act directly on mechanisms within the hair cells.

Cisplatin has been shown to inhibit protein synthesis at concentrations greater than required for inhibition of DNA synthesis (Harder and Rosenberg, '70). High concentrations in the cochlea may occur if cisplatin, like

the aminoglycosides, is sequestered in the cochlear fluids. Inhibition by cisplatin of an enzyme or enzymes vital to cellular processes could potentially result in death of the hair cell.

Recent evidence indicates that an investigation concerning cisplatin's effects on intracellular calcium regulation in hair cells may be warranted. Cellular calcium is known to be an important mediator in active transport systems, metabolic pathways, neurotransmitter release, and many other cellular functions. Nicotera et al. ('85) reported inhibition of plasma membrane Ca-ATPase activity in isolated rat hepatocytes incubated with cytotoxic concentrations of menadione and suggested that the inhibition by menadione was due to the oxidation of sulfhydryl groups critical for Ca-ATPase activity. Thor et al. ('82) demonstrated the formation of blebs on the surface structure of menadione-intoxicated hepatocytes which resembled blebs seen on hair cell stereocilia intoxicated with cisplatin. The formation of blebs on the hepatocytes were associated with reduced intracellular levels of calcium and thiol groups. Recently, sodium thiosulfate has been successfully utilized as a protective agent against cisplatin cytotoxicity (Howell et al., '82). If Ca-ATPase in the plasma membrane of hair cells is dependent on sulfhydryl groups, as seen with hepatocytes,

the binding of cisplatin to cellular sulfhydryl group would potentially inhibit plasma membrane Ca-ATPase and reduce intracellular levels of calcium. It can be suggested that outer hair cells, having a greater degree of metabolic activity, would be more vulnerable to damage than inner hair cells in the presence of abnormal intracellular calcium levels.

Summary

Cisplatin ototoxicity, as determined by measurements of Preyer reflex, cochlear compound action potential, and hair cell loss, was dose-dependent. Results from round window recordings of compound action potential demonstrated increases in latency and decreases in amplitude for guinea pigs treated with low doses of cisplatin while complete suppression of the action potential occurred with high doses. Outer hair cell destruction occurred sporadically throughout all turns of the cochlea with a strong prevalance in the basal turn and with no significant difference between outer hair cell rows. Various degrees of stereocilia distortion in both inner and outer hair cells was represented by separation, clumping, formation of blebs, and expulsion of hair cell content. Na,K-ATPase activity in cochlear lateral wall was not significantly different between treatment groups. These results support previous findings concerning cisplatin's toxic actions on

the organ of Corti and suggest that the pathogenesis of cisplatin ototoxicity is not directly related to Na,K-ATPase in cochlear lateral wall.

BIBLIOGRAPHY

BIBLIOGRAPHY

Aran, J-M 1981 Electrophysiology of cochlear toxicity. In: Aminoglycoside ototoxicity. C.J. Matz, S. Lerner and J.E. Hawkins, eds. Little, Brown and Co., Chicago, pp. 31-50.

Brown, R.D. 1975 Ethacrynic acid and furosemide: Possible cochlear sites and mechanisms of ototoxic action. Medikon 4:33-40.

Brown, R.D., and A.M. Feldman 1978 Pharmacology of hearing and ototoxicity. Ann-Rev. Pharmacol. Toxicol., 18:233-252.

Brownell, W.E., C.R. Bader, D. Bertrand, and Y. de Ribaupierre 1985 Evoked mechanical responses of isolated cochlear outer hair cells. Science, 227:194-196.

Calabresi, P., and R.E. Parks, Jr. 1980 Chemotherapy of neoplastic diseases. In: Goodman and Gilman's The Pharmalogcal Basis of Therapeutics. A.G. Gilman, L.S. Goodman, and A. Gilman, eds. MacMillan Publishing Co., New York, pp. 1298-1299.

Cody, A.R., and D. Robertson 1983 Variability of noise-induced damage in the guinea pig cochlea: Electro-physiological and morphological correlates after strictly controlled exposures. Hear. Res., 9:55-70.

Cody, A.R., and I.J. Russell 1985 Outer hair cells in the mammalian cochlea and noise-induced hearing loss. Nature, 3:15:662-665.

Daigneault, E.A., R.D. Brown, and J. Pruett 1968 Cochlear round window recorded response to acetylcholine and click stimulation following decentralization. Acta Otolaryngol., 66:10-16.

Daley-Yates, P.T., and D.C. McBrien 1982 The inhibition of renal ATPase by cisplatin and some bio-transformation products. Chem. Biol. Interact., 40:325-334.

Daley-Yates, P.T., and D.C. McBrien 1984 Cisplatin metabolites in plasma. A study of their pharmaco-kinetics and importance in the nephrotoxicity and antitumor activity of cisplatin. Biochem. Pharmacol., 15:3063-3070.

Davis, H. 1965 A model for transducer action in the cochlea. Sympos. Quant. Biol., 30:181-190.

Dorr, R.T., and W.L. Fritz 1980 Cisplatin. In: Cancer Chemotherapy Handbook. ed. Elsevier, New York, pp. 315-323.

Estrem, S.H., R.W. Babin, J.H. Ryu, and K.C. Moore 1981 Cis-diaminedichloroplatinum (II) ototoxicity in the guinea pig. Otolaryngol. Head Neck Surg., 89:638-645.

Falk, S.A., J.K. Haseman, and R. Klein 1973 Methyl mercury ototoxicity: A model for the surface preparation technique. Laryngoscope, 83: 1769-1782.

Fiske, C.H., and Y. Subba Row 1925 The calorimetric determination of phosphorus. J. Biol. Chem., 66:375-400.

Fleischman, R.W., S.W. Stadnicki, M.F. Ethier, and U. Schaeppi 1975 Ototoxicity of cis-dichlorodiamine-platinum (II) in the guinea pig. Toxicol. Appl. Pharmacol., 33:320-332.

Goldstein, M.H., and N. Y-S. Kiang 1958 Synchrony of neural activity in electric-responses evoked by transient acoustic stimuli. J. Acoust. Soc. Am., 30:107-112.

Goldstein, R.S., and G.H. Mayor 1983 The nephrotoxicity of cisplatin. Life Sci., 32:685-690.

Harder, H.C., and B. Rosenberg 1970 Inhibitory effects of antitumor platinum compounds on DNA, RNA, and protein synthesis in mammalian cells in vitro. Int. J. Cancer, 6:207-216.

Hawkins, J.E. 1976 Drug ototoxicity. In: Handbook of Sensory Physiology. W.D. Keidel and W.D. Neff, eds. Springer, New York, 3:707-748.

Hill, J.M. E. Loch, A. Machellan, N.O. Hill, A. Khan, and J.J. King 1975 Clinical studies of platinum coordination compounds in the treatment of various malignant diseases. Cancer Chemother. Rep., 59:647-659.

Hoff, D. Von, R. Schilsky, C.M. Reichert, R.L. Reddick, M. Rozencweig, R.C. Young, and F.M. Muggia 1979 Toxic effects of cis-dichlorodiamine platinum (II) in man. Cancer Treat. Rep., 63:1527-1531.

Howell, S.B., C.L. Pfeitle, W.E. Wung, R.A. Olshen, W.E. Lucas, J.L. Yon, and M. Green 1982 Intraperitoneal cisplatin with systemic thiosulfate protection. Ann. Intern. Med., 97:845-851.

Iinuma, I. 1969 Evaluation of adenosine triphosphatase activity in the stria vascularis and spiral ligament of normal guinea pigs. Laryngoscope, 77:141-158.

Kisiel, D.L., and R.P. Bobbin 1981 Miscellaneous ototoxic agents. In: Pharmacology of Hearing. R.D. Brown and E.A. Daigneault, eds. John Wiley & Sons, New York, pp. 231-270.

Komune, S., S. Asakuma, and J.B. Snow 1981 Pathophysiology of the ototoxicity of cis-diaminedichloroplatinum. Otolaryngol. Head Neck Surg., 89:275-282.

Konishi, T., B.N. Gupta, J. Prazma 1983 Ototoxicity of cis-dichloro-diamine platinum (II) in the guinea pig. Am. J. Otolaryngol., 4:18-26.

Kronester-Frei, A. 1979 The effect of changes in endolymphatic ion concentrations on the tectorial membrane. Hear. Res., 1:81-94.

Kwijpers, W., and S.L. Bonting 1969 Studies on (Na^+-K^+) activated ATPase. XXIV. Localization and properties of ATPase in the inner ear of the guinea pig. Biochim. Biophys. Acta, 173:477-487.

Lim, D.J., and W. Melnick 1971 Accoustic damage of the cochlea: A scanning and transmission electron microscope observation. Arch. Otolaryngol., 94:294-305.

Lim, D.J. 1976 Ultrastructural cochlear changes following acoustic hyperstimulation and ototoxicity. Arch. Otorhinolaryngol., 85:740-751.

Lim, D.J. 1972 Fine morphology of the tectorial membrane. Arch. Otolaryngol., 96:199-215.

Lippard, S.J. 1980 Binding of a platinum antitumor drug to its likely biological target. In: Inorganic Chemistry in Biology and Medicine. M.J. Constock, ed. A.C.S. Symposium Series 140, Washington, D.C., pp. 147-156.

Litterst, C.L., A.F. LeRoy, and A.M. Guarino 1979 Disposition and distribution of platinum following parenteral administration of cis-dichlorodiamine-platinum (II) to animals. Cancer Treat. Rep., 63:1485-1492.

Lowry, O., N. Rosebrough, A. Farr, R. Randall 1951 Protein measurement with the Folin phenol reagent. J. Biol. Chem., 193:265-275.

Mess, K. 1983 Ultrastructural localization of K^+-dependent ouabain-sensitive NPPase (Na-K-ATPase) in the guinea pig inner ear. Acta Otolaryngol., 95:227-289.

Merrin, C. 1978 Treatment of advanced bladder cancer with cis-diaminedichloro-platinum (II NSC 119875): a pilot study. J. Urol., 119:493-524.

Moroso, M.J., and R.L. Blair 1983 A review of cis-platinum ototoxicity. J. Otolaryngol., 12:365-369.

Nakai, Y., K. Konishi, K.C. Chang, K. Ohashi, N. Morisaki, Y. Minowa, and A. Morimoto 1982 Ototoxicity of the anticancer drug cisplatin. An experimental study. Acta Otolaryngol., 93:227-232.

Netter, F. 1970 Cross section of the cochlea. In: Otologic Diagnosis and Treatment of Deafness. D. Myers, W.D. Schlosser, R.J. Wolfson, R.A. Winchester, and N.H. Carmel. CIBA Clinical Symposia, 22:39.

Nicotera, P., M. Moore, F. Mirabelli, G. Bellomo, and S. Orrenius 1985 Inhibition of hepatocyte plasma membrane Ca^{2+}-ATPase activity by menadione metabolism and its restoration by thiols. FEBS, 181:149-153.

Piel, I.J., D. Meyer, C.P. Perlia, and V.I. Wolfe 1974 Effects of cis-diaminedichloroplatinum (NSC-119875) on hearing function in man. Cancer Chemother. Rep., 58:871-875.

Quick, C.A., A. Fish, and C. Brown 1973 The relationship between cochlea and kidney. Laryngoscope, 73:1469-1481.

Russell, I.J., and P.M. Sellick 1983 Low frequency characteristics of intracellularly recorded receptor potentials in guinea-pig cochlear hair cells. J. Physiol., 338:179-206.

Spoendlin, H. 1972 Innervation densities of the cochlea. Acta Otolaryngol., 73:235-248.

Tange, R.A., E,A.J.G. Conijin, and L.G.P.M. Von Zeijl 1982 The cortitoxic effects of cis-platinum in the guinea pig. Arch. Otorhinolaryngol., 237:17-26.

Thor, H., M.T. Smith, P. Hartzxzell, G. Bellomo, S.J. Jewell, and S. Orrenius 1982 The metabolism of menadione (2-methyl-1,4-naphthoquinone) by isolated hepatocytes. J. Biol. Chem., 257:12419-12425.

Wersall J. 1973 Problems and pitfalls in studies of cochlear hair cell pathology. In: The Auditory Periphery. A.R. Moller, ed. Academic Press, New York, pp. 235-256.

Williams, P.L., and R. Warwick 1980 Neurology. In: Gray's Anatomy. W.B. Saunders Co., Philadelphia, p. 1210.

Wit, H.P., and H.F. Nijdam 1984 Relationship of gross cochlear potentials to hair cell pathology in the waltzing guinea pig. Hear. Res., 15:159-171.

VITA

SARAH ELIZABETH BARRON

Personal Data:

Date of Birth: April 13, 1956
Place of Birth: Atlanta, Georgia
Marital Status: Married

Education:

East Tennessee State University, Johnson City, Tennessee: chemistry, B.S., 1978.

Quillen-Dishner College of Medicine at East Tennessee State University, Johnson City, Tennessee; biomedical science, M.S., 1982.

Quillen-Dishner College of Medicine at East Tennessee State University, Johnson City, Tennessee; biomedical science, Ph.D., 1985.

Professional Experience:

Teaching Assistantship in Chemistry, East Tennessee State University, 1978-1979.

Graduate Research Assistant in Pharmacology, Quillen-Dishner College of Medicine, East Tennessee State University 1980-1985.

Teaching Experience:

General Chemistry Laboratory, East Tennessee State University, 1978-1979.

Medical Pharmacology Laboratory, Quillen-Dishner College of Medicine, 1982-1985.

Medical Pharmacology, Quillen-Dishner College of Medicine, 1982-1984.

Pharmacology for Nurses, East Tennessee State University, 1985.

Societies and Professional Organizations:

Sigma Xi
Southeastern Regional Chapter of the Society of Toxicology
Southeastern Society of Pharmacology
Society of Neuroscience

Papers Delivered:

Barron, S.E. and E.A. Daigneault. Cisplatin Ototoxicity. Presented at the American Society for Pharmacology and Experimental Therapeutics, August, 1985.

Barron, S.E. and E.A. Daigneault. Cisplatin Ototoxicity in the Guinea Pig. Presented at the Southeastern Regional Chapter of the Societ of Toxicology, September, 1985.

Publications:

Hoover, D.B. and S.E. Barron 1982 Localization and Acetylcholinesterase Content of Vagal Efferent Neurons. Brain Res. Bul., 8:279-284.

Barron, S.E. and D.B. Hoover 1983 Localization of Acetylcholinesterase and Choline Acetyltransferase Activity in the Rat Pituitary Gland. Histochem. J., 15:1087-1098.